



PROTOCOL TO PRODUCTION OF wtGFP UNDER DIFFERENT PROMOTERS

1. Made a 10 mL pre-inoculation of DH5a :: puspA:GFP // pJ23101 using lysogenic broth (LB) containing 10 μ L de 35 mg/mL cloramphenicol. It was cultivated overnight at 37°C under 200 rpm.
2. Add to an Erlenmeyer flask of 500 mL containing 100 mL of LB supplemented with cloramphenicol (ratio of 1 μ L/mL of medium), about 5-10 mL of pre-inoculation. Incubate under 200 rpm, at 37°C until optical density of 0.5-0.6 (Near to 1,5-2h)
3. Distribute 1 mL of medium per well in a 96 deep well plate (rows A-C: vector puspA:GFP, D-E: vector pJ23101), centrifuge at 4000 rpm for 6 min, and repeat the operation once more.
4. Add 1 mL of culture media with different PEG6000 concentrations in each well, depending of plate column:
1 – PEG 0%; 2 – PEG 2%; 3 – PEG 4%;
4 – PEG 6%; 5 – PEG 8%; 6 – PEG 10%;
7 – PEG 12%; 8 – PEG 14%; 9 – PEG 16%;
10 – PEG 18%; 11 – PEG 20%; 12 – PEG 30%
5. Seal plate, making an aeration hole per well. Incubate plates for 4h at 37°C under 200 rpm.
6. Centrifuge for 10 min at 4,000 rpm at 4°C. Dispense supernatant and freeze pelleted cells at -20°C overnight.
7. Descongelar as células em gelo

8. Ressuspend cells in 180 μ L of Lysis Buffer without imidazole, and add 20 μ L of FastBreak Cell Lysis Reagent 10X (Promega). Change plate seal, carefully inversions for 15 min at room temperature.
9. Centrifuge lysate for 30 min at 4,000 rpm at 4°C
10. Recover supernatant and measure in fluoremeter as previously informed in protocol “STANDARD CURVE OF GFP FLUORESCENCE” or use a 96 wells black plate for optical reading and a Viktor equipment (Excitation: filter of 340nm, Emission: filter of 500nm).
11. Alternatively, make SDS-PAGE to compare controls (0% de PEG) and samples, as well as the different promoters.

Formula for different media containing PEG6000 in different concentrations

Conc %	LB2X	H2O	PEG50%	Chloram.	Volume total
0	5	5	0	10 uL	10 mL
2	5	4,6	0,4	10 uL	10 mL
4	5	4,2	0,8	10 uL	10 mL
6	5	3,8	1,2	10 uL	10 mL
8	5	3,4	1,6	10 uL	10 mL
10	5	3	2	10 uL	10 mL
12	5	2,6	2,4	10 uL	10 mL
14	5	2,2	2,8	10 uL	10 mL
16	5	1,8	3,2	10 uL	10 mL
18	5	1,4	3,6	10 uL	10 mL
20	5	1	4	10 uL	10 mL
30	4	0	6	10 uL	10 mL

Values are given in mL. Except antibiotics, which was given as microliters.

SDS-PAGE ORDER:

M_0%_2%_10%_30%_0%_2%_10%_30%
 UspA J23101